PCT/SG2004/000416 WO 2005/059177 3.2

Claims

15

- 1. A method for detecting SARS coronavirus nucleic acid in a sample comprising:
- A) amplifying a nucleic acid of said sample with a reverse 5 transcriptase and at least one primer specific for a NSP1 region of a SARS coronavirus to generate a nucleic acid ampification product; and
- B) analyzing said amplification product wherein detecting an expected nucleic acid amplification product indicates the 10 presence of SARS coronavirus nucleic acid in the sample.
 - The method of claim 1, wherein the at least one primer specific for the NSP1 region of the SARS coronavirus is a primer consisting essentially of a sequence selected from the group consisting of SEQ ID NOs: 3, 4, 6, 7, 9 and 10.
- The method of claim 1, wherein the at least one primer specific for the NSP1 region of the SARS coronavirus is a primer consisting of a sequence selected from the group 20 consisting of SEQ ID NOs: 3, 4, 6, 7, 9 and 10.
 - 4. The method of claim 1, wherein a primer having the nucleotide sequence of SEQ ID NO: 3 and a primer having the nucleotide sequence of SEQ ID NO: 4 are used and the expected nucleic acid amplification product is detected as a polynucleotide that is 352 nucleotides long.
- The method of claim 1, wherein a primer having the nucleotide sequence of SEQ ID NO: 6 and a primer having the 30 nucleotide sequence of SEQ ID NO: 7 are used and the expected nucleic acid amplification product is detected as a polynucleotide that is 157 nucleotides long.

- 6. The method of claim 1, wherein a primer having the nucleotide sequence of SEQ ID NO: 9 and a primer having the nucleotide sequence of SEQ ID NO: 10 are used and the expected nucleic acid amplification product is detected as a
- 5 expected nucleic acid amplification product is detected as a polynucleotide that is 77 nucleotides long.
 - (7. The method of any one of claims 1 to 6, wherein said nucleic acid amplification product is analyzed by chromatography.

10

- 8. The method of claim 7 wherein the nucleic acid amplification is performed by a polymerase chain reaction.
- 9. A method for detecting SARS coronavirus nucleic acid in a sample comprising:
 - A) amplifying a nucleic acid of said sample with a reverse transcriptase and a first primer having the sequence of SEQ ID NO: 9 and a second primer having the sequence of SEQ ID
- 20 NO: 10 to generate a nucleic acid amplification product;
 - B) detecting said nucleic acid amplification product by specific hybridization of a probe having the sequence of SEQ ID NO: 11;
- wherein detection of a specifically hybridizing amplified 25 nucleic acid fragment indicates the presence of SARS coronavirus nucleic acid in the sample.
 - 10. The method of claim 9, in which the nucleic acid amplification is performed by a polymerase chain reaction.
 - 11. A method for detecting the presence of SARS coronavirus nucleic acid in a sample, comprising:

PCT/SG2004/000416

WO 2005/059177

i) amplifying nucleic acids present in the sample using a forward primer and a reverse primer selective for the region of the SARS coronavirus genome from nucleotide 6652 to nucleotide 7003, said primers having a certain primer length in nucleotides and being separated by a separation length that is a certain number of nucleotides, to obtain an amplification product;

34

- ii) determining the length of the amplification product in nucleotides;
- iii) the presence of an amplification product having a length in nucleotides that is the sum of the forward primer length, the reverse primer length and the separation length indicating the presence of SARS coronavirus nucleic acid in the sample.

- 12. The method of claim 12, in which the nucleic acid amplification is performed by a polymerase chain reaction.
- 13. A method for detecting the presence of SARS coronavirus20 nucleic acid in a sample, comprising:
 - i) amplifying nucleic acids present in the sample using a forward primer and a reverse primer selective for the region of the SARS coronavirus genome from nucleotide 6652 to nucleotide 7003 to obtain an amplification product; and
- 25 ii) detecting the amplification product by specific hybridization of a probe having a nucleotide sequence of at least 18 contiguous nucleotides of the portion of the SARS coronavirus genome from nucleotide 6652 to 7003; specific hybridization of the probe to the amplification product indicating the presence of SARS nucleic acid in the sample.

- 14. The method of claim 13, in which the nucleic acid amplification is performed by a polymerase chain reaction.
- 15. A method for detecting the presence of SARS coronavirus 5 nucleic acid in a sample, comprising:
 - i) amplifying nucleic acids present in the sample using a forward primer and a reverse primer selective for the region of the SARS coronavirus genome from nucleotide 4609 to nucleotide 4765, said primers having a certain primer length in nucleotides and being separated by separation length that is a certain number of nucleotides, to obtain an amplification product;
 - ii) determining the length of the amplification product in nucleotides;
- 15 iii) the presence of an amplification product having a length in nucleotides that is the sum of the forward primer length, the reverse primer length and the separation length indicating the presence of SARS coronavirus nucleic acid in the sample.

20

- 16. The method of claim 15, in which the nucleic acid amplification is performed by a polymerase chain reaction.
- 17. A method for detecting the presence of SARS coronavirus 25 nucleic acid in a sample, comprising:
 - i) amplifying nucleic acids present in the sample using a forward primer and a reverse primer selective for the region of the SARS coronavirus genome from nucleotide 4609 to nucleotide 4765 to obtain an amplification product; and
- ii) detecting the amplification product by specific hybridization of a probe having a nucleotide sequence of at least 18 contiguous nucleotides of the portion of the SARS coronavirus genome from nucleotide 4609 to 4765;

WO 2005/059177 PCT/SG2004/000416

36

specific hybridization of the probe to the amplification product indicating the presence of SARS coronavirus nucleic acid in the sample.

5 18. The method of claim 17, in which the nucleic acid amplification is performed by a polymerase chain reaction.

10

25

- 19. A SARS coronavirus detection kit comprising at least one primer for a nucleic acid amplification reaction selected from the group consisting of SEQ ID NOs: 3, 4, 6, 7, 9 and 10.
- 20. The SARS coronavirus detection kit according to claim
 19, that comprises at least one pair of primers selected
 15 from the group consisting of a primer having the sequence of
 SEQ ID NO: 3 and a primer having the sequence of SEQ ID NO:
 4, a primer having the sequence of SEQ ID NO: 6 and a primer
 having the sequence of SEQ ID NO: 7, and a primer having the
 sequence of SEQ ID NO: 9 and a primer having the sequence of
 20 SEQ ID NO: 10.
 - 21. The SARS coronavirus detection kit according to claim 19 or 20, that also comprises a SARS coronavirus genomic nucleic acid, or at least a portion thereof comprising the NSP1 region.
 - 22. The SARS coronavirus detection kit according to claim 21, in which the SARS coronavirus genomic nucleic acid has the nucleotide sequence of SEQ ID NO: 1, or the RNA equivalent thereof.
 - 23. Use of an oligonucleotide having a sequence selected from the group consisting of SEQ ID NOs: 3, 4, 6, 7, 9, 10

and 11, in a method for detecting the presence of SARS coronavirus nucleic acids in a sample.